

REMARKS

The Examiner's action in withdrawing claims 3 and 4 from further consideration has been noted.

The Examiner's indication of the allowability of original claims 8, 9, 13 and 14 has been noted and these claims have been rewritten as claims 22-25 to include all of the limitations of original claims 8, 9, 13 and 14 and to avoid the formal objections. In particular, new claim 22, which combines original claims 1 and 8, the term "the" has been deleted in connection with the terms "muscular" and "contracting". The term SEQ ID NO 1 has been added to the listed peptide. The "2" in the pentapeptide has been set forth as a subscript and in claims 24 and 25, which are based on original claims 13 and 14, the term "SEQ ID NO 1" has been added. For these reasons, favorable consideration of claims 22-25 is requested.

The approval of the Sequence Listing has been noted.

In response to the objection to the drawings, a replacement sheet is being filed with this amendment that substitutes the English WORD "CONTRACTION" for the Italian word "CONTRAZIONE". For this reason, it is requested that the objection to the drawings be withdrawn.

In response to the objection to the specification that no Brief Description of the Drawings is present in the specification, the specification has been amended to insert the required description. In addition, the specification has been amended to insert the SEQ ID NOS where appropriate.

Claims 5-7 and 10 were rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that the applicant regards as the invention.

Reconsideration is requested.

Claims 5 and 6 have been canceled and the SEQ ID NOS have been inserted in claims 13 and 14. The number 2 on the NH₂ group has been rewritten as a subscript. Claims 8 and 9 have been canceled and claims 10, 12 and 19 have been amended to adopt the Examiner's kind suggestions for revision. For these reasons, it is requested that this ground of rejection be withdrawn.

In the Office Action, claims 1, 2 5-7, 9, 15, 19 and 21 were rejected under 35 U.S.C. §102(a) and (c) as being anticipated by Lintner et al., U.S. 2004/0120918 (Lintner '918) as evidenced by Lintner et al., U.S. 2004/0132667 (Lintner '667).

Claim 16 was rejected under 35 U.S.C. §103(a) as being unpatentable over Lintner et al., U.S. 2004/0120918 (Lintner '918) as evidenced by Lintner et al. ('667). and claims 9, 11 and 12 were rejected over the same references in combination with Sojka, Patt and Renault.

Claims 16-18 were rejected under 35 U.S.C. §103(a) as being unpatentable over Lintner et al., U.S. 2004/0120918 (Lintner '918) as evidenced by Lintner '667 and Laversanne et al.

Claim 20 was rejected under 35 U.S.C. §103(a) as being unpatentable over Lintner et al., U.S. 2004/0120918 (Lintner '918) as evidenced by Lintner et al. and Donovan et al.

Claims 1, 2, 9, 11, 15, 19 and 21 reaction as being anticipated under Renault.

Reconsideration is requested.

Claim 1 has been amended to specify that the cosmetic composition comprises a specific type of a dipeptide or a specific pentapeptide or a mixture thereof with a magnesium, sodium or potassium salt or a mixture thereof where the sodium and potassium salts are derived from a natural source. Support for this amendment is found in aoriginal claims 2, 4 and 5 and in original, claims 10, 11 and 12. In addition, the specification at page 9, line 15 to page 11, line 2 and page 12, line 23 to page 13, line 25.

The Lintner '918 patent discloses a composition for treating wrinkles comprising Calmosensine[®] (an acetyl tyrosine-arginine-1 cetyl ester), sodium hydroxide and potassium sorbate, that according to the Examiner correspond to the micro-element disclosed by the Applicant.

Potassium sorbate is a conventional preservative used in cosmetic and dietary field and sodium hydroxide is usually employed as pH corrector in the manufacture of cosmetics. Both of these materials are synthetic substances which do not make obvious the use of potassium and sodium from a natural source as pointed out in amended claim 1.

The Applicant clearly disclosed that according to one preferred embodiment of the invention the source of potassium and sodium salts is a natural source, such as an aqueous based extract of anise (see pages 12-13 of the original text and claim 10). Such extract is rich in sodium and potassium ions. Such natural substances are used as carrier of active principles, in the present case of potassium and sodium ions. As noted above, claim 1[~] has been amended to point out this feature in accordance with page 13, line 14.

The Sokja pstent is limited to a mention of potassium gluconate as a skin conditioning agent. No peptides are disclosed by this reference. Patt is limited to disclosing the use of potassium gluconate as a skin protectant and fails to mention the use of that material in combination with a peptide. Renault is concerned with the use of a peptide and a magnesium compound and fails to mention the use of sodium and potassium from a natural source in combination with a peptide. Donovan is limited to a botulin toxin application which involves a technique of injection that is critical because of the highly toxic nature of the botulin toxin. There is nothing in Donovan that can be considered a general application method for any other anti-wrinkle preparation.

The collective teachings of the cited references do not make obvious the use of the recited dipeptide and or pentapeptide with the naturally derived sodium and potassium salts or the magnesium salt as defined in amended claim 1. Therefore, the rejections of record are avoided by this Amendment and it is requested that these rejections be withdrawn.

Attached to this Amendment are Exhibit A and Exhibit B which provide comparative experimental data that compares the myorelaxant activity of Lintner's composition and the composition according to the invention containing potassium and sodium from a natural source, namely, anise extract.

Study 1 concerns the different myorelaxant activity of the following mixtures:

- Mix 1: dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + sodium potassium association (from anise extract) corresponding to the composition according to claims 9-10
- Mix 2: dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + sodium hydroxide + potassium sorbate corresponding to the composition disclosed by Lintner et al.

The test results show that Mix 1 has a more potent and long lasting inhibitory effect on muscle contraction in comparison to the prior art composition Mix 2 (Lintner et al.). Such a result is clearly unpredictable and demonstrates the advantageous and unobvious effects of using an association of micro-elements from a natural source instead of synthetic potassium sorbate and sodium hydroxide in combination with the dipeptide.

Study 2 is a further comparative study of the different myorelaxant activities of the following mixtures:

- Mix 2: dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + sodium hydroxide + potassium sorbate corresponding to the composition disclosed by Lintner et al.
- Mix 3: dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + magnesium gluconate corresponding to the composition according to claim 11.

The following results were obtained:

- The Mix 3 composition has a higher myorelaxant effect in comparison to the prior art composition Mix 2 (Lintner et al.). Again, such a result is clearly unpredictable and demonstrates the advantageous effect of using magnesium gluconate together with the dipeptide instead of synthetic potassium sorbate and sodium hydroxide.

An early and favorable action is earnestly solicited.

Respectfully submitted,



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Study 1- Serial No. 10/583,816

N. 1 Effect of 2 associations of compounds on contraction frequency of muscle fibers

INTRODUCTION

Potential myorelaxant effects of 2 associations of compounds:

- Mix 1, Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + SODIUM-POTASSIUM (Pimpinella anisum extract) and
- Mix 2, Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + SODIUM HYDROXIDE + POTASSIUM SORBATE

were investigated on an *in vitro* model of contractile innervated muscle fibers.

These 2 associations both contained test compound Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) for which a myorelaxant activity was shown in previous study GT090420.

Striated muscle cells used in this model were human primary myoblasts which fuse to form primary myotubes after 10 days of culture. Addition of a rat explant of spinal cord containing motoneurons and responsible for muscle cell innervation, allows recreating a motor end plate after 20 days of co-culture.

This heterologous system (human myoblasts + rat motoneurons) is functional as formation of motor end plate occurs with muscle fibers contractions. Contraction frequency can be then recorded.

MATERIALS AND METHODS

Biological models

Muscle cells

- Cell type: Human myoblasts, BIOalternatives reference: Mu2b
- Culture conditions: 37°C, 5% CO₂
- Proliferation medium: MEM 65% (v/v) + M199 25% (v/v) supplemented with Antibiotics (Penicillin 50 U/ml - Streptomycin 50 µg/ml)
L-glutamine 2 mM
Epidermal Growth Factor (EGF) 10 ng/ml
Fibroblast Growth Factor (FGF) 2 ng/ml
Insulin 10 µg/ml
Foetal Calf Serum (FCS) 10% (v/v)

Spinal cord explant / muscle fibers co-culture

- Cell type: Primary myotubes
Spinal cord explant containing primary motoneurons
- Culture conditions: 37°C, 5% CO₂
- Innervation medium: MEM 65% (v/v) + M199 25% (v/v) supplemented with Antibiotics (Penicillin 50 U/ml - Streptomycin 50 µg/ml)

L-glutamine 2 mM

Insulin 5 µg/ml

FCS 5% (v/v)

Compounds and test mixes

Test compound	Aspect/Storage	Test concentrations
Dipeptide (Acetyl Dipeptide-1 Cetyl Ester)	Powder Storage at RT	Used at 5 ppm in mixes
Potassium Sorbate	Grain Storage at +4°C protected from light and moisture	Used at 0.16 and 0.8 mg/ml in mix 2
Sodium Hydroxide	Pellets Storage at +4°C protected from light and moisture	Used at 0.08 and 0.4 mg/ml in mix 2
Sodium-Potassium (Pimpinella anisum extract)	Powder Storage at +4°C protected from light and moisture	Used at 0.4 and 2 mg/ml in mix 1

Mix	Test concentrations
Mix 1: Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + Sodium-Potassium (Pimpinella anisum extract)	5 ppm + 0,4 mg/ml and 5 ppm + 2 mg/ml
Mix 2: Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + Potassium Sorbate + Sodium Hydroxide	5 ppm + 0,16 mg/ml + 0,08 mg/ml and 5 ppm + 0,8 mg/ml + 0,4 mg/ml

Culture treatment and contraction frequency analysis

Co-cultures were observed with an inverted microscope equipped with a video camera (acquisition of video sequences for 30 seconds) and a slaved motorized platform. Coordinates of observed fields containing contractile muscle fibers were recorded and allowed automatically repositioning.

After 20 days of co-culture, contraction frequency measurements were performed before contact (control) with test compounds or reference (carisoprodol at 1 mM) and after each incubation period (1 minute, 2 hours and 24 hours) in the same well (respectively for each compound) and on the same fibers selected for the measurement of control.

- Before compound contact, the frequency of the number of contractions was evaluated for 30 second to obtain the basal frequency (control). Immediately after that, the contraction frequency after 1 minute of contact with test compound was evaluated in order to assess immediate effects.
- After 2 hours of incubation, the frequency of contraction was evaluated for a new period of 30 seconds.
- After 24 hours of incubation, cells were observed to check co-culture integrity. Due to glucose

consumption and to avoid consequent decrease of contraction frequency, culture medium was complemented with a solution of concentrated glucose (4 g/l). One minute after glucose addition, the number of contractions was evaluated on a new period of 30 seconds.

- The culture medium was then removed and cells were washed. Innervation medium without test compounds or reference was then added and cells were incubated for 24 hours = 24 hours of recovery. After this last incubation, the concentrated glucose solution was added, as mentioned above, and then the number of contractions was evaluated on a period of 30 seconds. Measurement performed at 48 hours thus corresponded to 24 hours of contact with test compounds + 24 hours of recovery time.

Data management and result interpretation

For each experimental condition, the number of contractions was expressed as percentage of the basal frequency.

The following parameters were used to analyze results and define the effect of test compounds:

Number of contractions	Effect
< -25%*	Stimulation
From -25% to 25%	No effect
From 25% to 75%	Myorelaxant effect
> 75%	Inhibition

RESULTS

Contraction frequency analysis

Table 2

Under these experimental conditions, contraction frequencies of muscle fibers of the control condition were highly fluctuating between different times of incubation and between each replicate. However, even contraction frequencies tended to increase over time when compared to basal frequencies, no inhibitory effect was observed in this control condition. This fluctuation is frequently observed with this complex *in vitro* assay involving two different primary cells and a functional endpoint.

An inhibitory effect of carisoprodol (tested at 1 mM) was observed after 1 minute of incubation on contraction frequency of the muscle fibers. After 2 and 24 hours of incubation, carisoprodol induced a total inhibition of muscle fiber contraction. At 48 hours, including 24 hours of recovery in absence of carisoprodol, contraction frequency tended to return toward basal value. These results were expected and validated the assay.

After 1 minute of incubation with Mix 1, at the highest test concentration, a total inhibition of muscle fiber contraction was observed. This total inhibitory effect was still observed after 2 and 24 hours of incubation. Moreover, even after 48 hours of incubation including 24 hours of recovery in absence of the mix, the inhibitory effect of this mix on muscle contraction remained total.

At the second test concentration, Mix 1 also presented an inhibitory effect on muscle fiber contraction frequency after 1 minute and 2 hours of incubation. However, this effect was less pronounced compared to the effect of Mix 1 at highest test concentration. These results thus suggest a dose-dependent effect of sodium-potassium contained in Mix 1. After 24 hours of incubation, this inhibitory effect could no longer be observed. Surprisingly, after 48 hours of incubation including 24 hours of recovery in absence of the mix, a total inhibition of muscle contraction was also observed. These results indicate a long-term inhibitory effect on muscle contraction for Mix 1 at both test concentrations.

After 1 minute of incubation with Mix 2, a total decrease of muscle fiber contractions was shown. However, this effect was due to a potent toxic effect rather than a specific inhibitory

effect since the application of this mix at highest test concentration resulted in few seconds in retraction and dislocation of the muscle fibers (as it may be visualized in corresponding video). Therefore no muscle contraction could be observed in later recording times. Thus the effect of Mix 2 at this test concentration could not be interpreted because of its toxic effect on muscle fiber.

At second test concentration, Mix 2 presented weak and moderate inhibitory effect on muscle contraction frequency after 1 minute and 2 hours of incubation, respectively. After 24 hours of incubation, this effect was no longer observed and muscle contraction frequency returned to basal frequency after 48 hours of incubation including 24 hours of recovery in absence of the mix.

CONCLUSION

Under these experimental conditions, Mix 1, corresponding to the association of Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) and sodium potassium, presented a potent and persistent inhibitory effect on muscle contraction. On the other hand Mix 2, corresponding to the association of Dipeptide (Acetyl Dipeptide-1 Cetyl Ester), potassium sorbate and sodium hydroxyde, presented a moderate and transitory myorelaxant effect on muscle fiber contraction frequency. Thus the myorelaxant effect of Mix 1 was more potent than myorelaxant effect of Mix 2.

Table 2: Effects of mix of compounds Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + SODIUM-POTASSIUM (Pimpinella anisum extract) and Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + SODIUM HYDROXIDE + POTASSIUM SORBATE on the modulation of muscle fiber contraction frequency.

Treatment	Concentration	Before contact with compound	After 1 minute of contact				After 2 hours of contact				After 24 hours of contact				48 hours (after 24 hours of recovery)			
			Number of contractions	% decrease of number of contractions	Mean (%)	sem (%)	Number of contractions	% decrease of number of contractions	Mean (%)	sem (%)	Number of contractions	% decrease of number of contractions	Mean (%)	sem (%)	Number of contractions	% decrease of number of contractions	Mean (%)	sem (%)
Control	-	36	83	-134			17	54			40	-11			69	-93		
		27	69	-158		-79	67	20	26	40	-213	-74	70	29	-9	-29	32	
		120	54	55				72	40			118	2			103	14	
Carisoprodol	1 mM	40	29	27			0	100			0	100			48	-20		
		53	0	100		43	29	0	100	100	0	100	0	11	79	38	30	
		39	38	4				0	100			0	100			18	54	
Mix 1	N-ACETYL-TYR-ARG-HEXADECYL ESTER - 5 ppm + SODIUM-POTASSIUM - 0.4 mg/ml	27	0	100			1	96			72	-165			0	100		
		27	11	59		86	14	0	100	96	2	0	100	-13	79	0	100	0
		109	0	100				9	92			80	27			0	100	
	N-ACETYL-TYR-ARG-	60	0	100		100	0	100	100	0	0	100			0	100	100	0

MIX 2	HEXADECYL ESTER - 5 ppm+	72	0	100				0	100			0	100				
	SODIUM- POTASSIUM - 2 mg/ml	16	0	100				0	100			0	100				
	N-ACETYL- TYR-ARG- HEXADECYL	42	26	38				40	5			48	-13			40	6
	ESTER - 5 ppm +	18	26	-49		8	28	9	49	40	19	72	-309	-78	119	37	-111
	POTASSIUM SORBATE - 0.16 mg/ml + SODIUM HYDROXIDE - 0.08 mg/ml	99	65	35				32	68			12	88			7	93
	N-ACETYL- TYR-ARG- HEXADECYL ESTER - 5 ppm +	103	0	100				0	100			0	100			0	100
	POTASSIUM SORBATE - 0.8 mg/ml + SODIUM HYDROXIDE - 0.4 mg/ml	44	0	100				0	100			0	100			0	100
		41	0	100		100	0	0	100	100	0	0	100	100	0	0	100
		65	0	100				0	100			0	100			61	7
		72	0	100				0	100			0	100			49	33

Study No. 2 Serial No. 10/583,816

N.2 Effect of 2 associations of compounds on contraction frequency of muscle fibers

INTRODUCTION

Potential myorelaxant effects of 2 associations of compounds:

- Mix 2, Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + SODIUM HYDROXIDE + POTASSIUM SORBATE and
- Mix 3, Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + MAGNESIUM GLUCONATE

were investigated on an *in vitro* model of contractile innervated muscle fibers.

These 2 associations both contained test compound Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) for which a myorelaxant activity was shown in previous study GT090420.

Striated muscle cells used in this model were human primary myoblasts which fuse to form primary myotubes after 10 days of culture. Addition of a rat explant of spinal cord containing motoneurons and responsible for muscle cell innervation allows recreating a motor end plate after 20 days of co-culture.

This heterologous system (human myoblasts + rat motoneurons) is functional as formation of motor end plate occurs with muscle fibers contractions. Contraction frequency can be then recorded.

MATERIALS AND METHODS

Biological models

Muscle cells

- Cell type: Human myoblasts, BIOalternatives reference: Mu2b
- Culture conditions: 37°C, 5% CO₂
- Proliferation medium: MEM 65% (v/v) + M199 25% (v/v) supplemented with Antibiotics (Penicillin 50 U/ml - Streptomycin 50 µg/ml) L-glutamine 2 mM Epidermal Growth Factor (EGF) 10 ng/ml Fibroblast Growth Factor (FGF) 2 ng/ml Insulin 10 µg/ml Foetal Calf Serum (FCS) 10% (v/v)

Spinal cord explant / muscle fibers co-culture

- Cell type: Primary myotubes Spinal cord explant containing primary motoneurons
- Culture conditions: 37°C, 5% CO₂
- Innervation medium: MEM 65% (v/v) + M199 25% (v/v) supplemented with Antibiotics (Penicillin 50 U/ml - Streptomycin 50 µg/ml) L-glutamine 2 mM

Insulin 5 µg/ml

FCS 5% (v/v)

Compounds and test mixes

Test compound	Aspect/Storage	Test concentrations
Dipeptide (Acetyl Dipeptide-1 Cetyl Ester)	Powder Storage at RT	Used at 5 ppm in mixes
Potassium Sorbate	Grain Storage at +4°C protected from light and moisture	Used at 0.16 and 0.8 mg/ml in mix 2
Sodium Hydroxide	Pellets Storage at +4°C protected from light and moisture	Used at 0.08 and 0.4 mg/ml in mix 2
Magnesium Gluconate	Powder Storage at +4°	Used at 4 and 20 mg/ml in mix 3

Mix	Test concentrations
Mix 2: Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + Potassium Sorbate + Sodium Hydroxide	5 ppm + 0.16 mg/ml + 0.08 mg/ml and 5 ppm + 0.8 mg/ml + 0.4 mg/ml
Mix 3 : Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + Magnesium Gluconate	5 ppm + 4 mg/ml and 5 ppm + 20 mg/ml

Culture treatment and contraction frequency analysis

Co-cultures were observed with an inverted microscope equipped with a video camera (acquisition of video sequences for 30 seconds) and a slaved motorized platform. Coordinates of observed fields containing contractile muscle fibers were recorded and allowed automatically repositioning.

After 20 days of co-culture, contraction frequency measurements were performed before contact (control) with test compounds or reference (carisoprodol at 1 mM) and after each incubation period (1 minute, 2 hours and 24 hours) in the same well (respectively for each compound) and on the same fibers selected for the measurement of control.

- Before compound contact, the frequency of the number of contractions was evaluated for 30 second to obtain the basal frequency (control). Immediately after that, the contraction frequency after 1 minute of contact with test compound was evaluated in order to assess immediate effects.

- After 2 hours of incubation, the frequency of contraction was evaluated for a new period of 30 seconds.

- After 24 hours of incubation, cells were observed to check co-culture integrity. Due to glucose consumption and to avoid consequent decrease of contraction frequency, culture medium was complemented with a solution of concentrated glucose (4 g/l). One minute after glucose addition, the number of contractions was evaluated on a new period of 30 seconds.

- The culture medium was then removed and cells were washed. Innervation medium without test compounds or reference was then added and cells were incubated for 24 hours = 24 hours of recovery. After this last incubation, the concentrated glucose solution was added, as mentioned above, and then the number of contractions was evaluated on a period of 30 seconds. Measurement performed at 48 hours thus corresponded to 24 hours of contact with test compounds + 24 hours of recovery time.

Data management and result interpretation

For each experimental condition, the number of contractions was expressed as percentage of the basal frequency.

The following parameters were used to analyze results and define the effect of test compounds:

Number of contractions	Effect
< -25%*	Stimulation
From -25% to 25%	No effect
From 25% to 75%	Myorelaxant effect
> 75%	Inhibition

RESULTS

Contraction frequency analysis

Table 2

Under these experimental conditions, contraction frequencies of muscle fibers of the control condition were highly fluctuating between different times of incubation and between each replicate. However, even contraction frequencies tended to increase over time when compared to basal frequencies, no inhibitory effect was observed in this control condition. This fluctuation is frequently observed with this complex *in vitro* assay involving two different primary cells and a functional endpoint.

An inhibitory effect of carisoprodol (tested at 1 mM) was observed after 1 minute of incubation on contraction frequency of the muscle fibers. After 2 and 24 hours of incubation, carisoprodol induced a total inhibition of muscle fiber contraction. At 48 hours, including 24 hours of recovery in absence of carisoprodol, contraction frequency tended to return toward basal value. These results were expected and validated the assay.

After 1 minute of incubation with Mix 2, a total decrease of muscle fiber contractions was shown. However, this effect was due to a potent toxic effect rather than a specific inhibitory effect since the application of this mix at highest test concentration resulted in few seconds in retraction and dislocation of the muscle fibers (as it may be visualized in corresponding video). Therefore no muscle contraction could be observed in later recording times. Thus the effect of Mix 2 at this test concentration could not be interpreted because of its toxic effect on muscle fiber.

At second test concentration, Mix 2 presented weak and moderate inhibitory effect on muscle contraction frequency after 1 minute and 2 hours of incubation, respectively. After 24 hours of incubation, this effect was no longer observed and muscle contraction frequency returned to basal frequency after 48 hours of incubation including 24 hours of recovery in absence of the mix.

Treatment with Mix 3 at both test concentrations, resulted in total inhibition of muscle fiber contractions after 1 minute, 2 hours and 24 hours of incubation. After 48 hours of incubation, including 24 hours of recovery in absence of the mix, the contraction frequency tended to return toward basal contraction frequency when the mix was tested at highest test concentration. At second test concentration, this tendency was more pronounced since a total recovery of muscle contraction frequency was observed.

CONCLUSION

Under these experimental conditions, Mix 3, corresponding to the association of Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) and magnesium gluconate, presented a potent myorelaxing effect on muscle contraction. On the other hand Mix 2, corresponding to the association of Dipeptide (Acetyl Dipeptide-1 Cetyl Ester), potassium sorbate and sodium hydroxyde, presented a moderate and transitory myorelaxant effect on muscle fiber contraction frequency. Thus the myorelaxant effect of Mix 3 was more potent than myorelaxant effect of Mix 2.

Table 2: Effects of mix of compounds Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + SODIUM HYDROXIDE + POTASSIUM SORBATE and Dipeptide (Acetyl Dipeptide-1 Cetyl Ester) + MAGNESIUM GLUCONATE on the modulation of muscle fiber contraction frequency

Treatment	Concentration	Before contact with compound	After 1 minute of contact				After 2 hours of contact				After 24 hours of contact				48 hours (after 24 hours of recovery)			
			Number of contractions	% decrease of number of contractions	Mean (%)	SEM (%)	Number of contractions	% decrease of number of contractions	Mean (%)	SEM (%)	Number of contractions	% decrease of number of contractions	Mean (%)	SEM (%)	Number of contractions	% decrease of number of contractions	Mean (%)	SEM (%)
Control		36	83	-134	-79		17	54	40		40	-11	70		69	-93		
		27	68	-158			20	26			83	-213			29	-9		
		120	54	55			72	40			118	2			103	14		
Carisoprodol	1 mM	40	29	27			0	100			0	100			48	-20		
		53	0	100			0	100			0	100			11	79		
		39	38	4	43		0	100	100		0	100	100		18	54	38	
		72	0	100			0	100			0	100			0	100		
		16	0	100			0	100			0	100			0	100		

MIX 2	N-ACETYL- TYR-ARG- HEXADECY LESTER - 5 ppm + POTASSIU M	42	26	38	8	28	40	5	40	19	48	-13	-78	119	40	37	6	-4	59
	SORBATE - 0.16 mg/ml + SODIUM HYDROXID E - 0.08 mg/ml	99	65	35			32	68			12	88				7	93		
	N-ACETYL- TYR-ARG- HEXADECY LESTER - 5 ppm + POTASSIU M	103	0	100			0	100			0	100				0	100		
	SORBATE - 0.8 mg/ml + SODIUM HYDROXID E - 0.4 mg/ml	44	0	100			0	100			0	100				0	100		
	N-ACETYL- TYR-ARG- HEXADECY LESTER - 5 ppm + MAGNESIU M	63	0	100			0	100			0	100				23	84		
	GLUCONAT E - 4 mg/ml	44	0	100			0	100			0	100				51	-17		
	N-ACETYL- TYR-ARG- HEXADECY LESTER - 5 ppm + MAGNESIU M	27	0	100			0	100			0	100				88	-232		
	GLUCONAT E - 20 mg/ml	52	0	100			0	100			0	100				39	24		
	N-ACETYL- TYR-ARG- HEXADECY LESTER - 5 ppm + MAGNESIU M	65	0	100			0	100			0	100				61	7		
	GLUCONAT E - 20 mg/ml	72	0	100			0	100			0	100				49	33		